Genetically Engineering the Ideal Gut Microbe: Using Synthetic Biology to Make Folate Factories Out of *Escherichia coli*

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Folate, a term which encompasses the various forms of the vitamin B9, is an essential vitamin involved in everyday cell functions such as DNA replication. Unable to naturally produce folate, humans must obtain it from vegetables or folate-supplements. In regions with little or no access to these foods, folate deficiencies can cause serious birth defects. One possible solution to alleviate the effects of folate deficiency is to engineer a strain of gut microbes to produce bioavailable folate directly in the colon. A total of four heterologous genes, two from the folate biosynthesis gene cluster and two from the pABA synthesis pathway, were tested. Using standardized genetic sequences, folate biosynthesis genes extracted from the *Lactoccocus lactis* genome were cloned into Biobricks ™ plasmids, transformed into *Escherichia coli* and overexpressed. The effects of overexpression were measured in terms of total folate and para-aminobenzoic acid (pABA) levels. PABA, an intermediate in folate synthesis, was detected using high performance liquid chromatography (HPLC). Folate detection was achieved via a microbiological assay. A measurable increase in folate production in *E.coli* would be a proof-of-concept for both the feasibility of engineering overproduction of folate in *E.coli* as well as using standardized genetic components to do so.